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USE OF SYNTHETIC PEPTIDES ANTI-IDIOTYPES FOR CONTROLLING HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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We are examining the correlation between the presence of antibodies in sera which recognize synthetic peptides whose sequences correspond to the principal neutralizing determinant of HIV-1 and the ability of these sera to neutralize homologous HIV-1 isolates *in vitro*. Sera from infected individuals from the United States and Tanzania were examined. Our data indicates that there is no strong correlation between the presence of anti-V3 antibodies in human sera and viral neutralizing activity. In addition, a composite peptide representing conserved regions within the V3 loop of HIV-1 gp120 has been produced and is currently being used to generate monoclonal and polyclonal anti-peptide antibodies in mice and rabbits, respectively. These antibodies are being tested for their ability to recognize and neutralize a panel of HIV-1 isolates. Recent reports indicate that regional isolates of HIV-1 may be present throughout the world. Differences in epidemiologic patterns suggest that these isolates may differ in their levels of virulence. We are therefore isolating primary HIV-1 isolates from infected individuals from the United States and Tanzania. We currently have 12 primary isolates from the United States and 2 from Tanzania. These HIV-1 isolates will be tested for differences in cell culture characteristics as well as for phenotypic differences.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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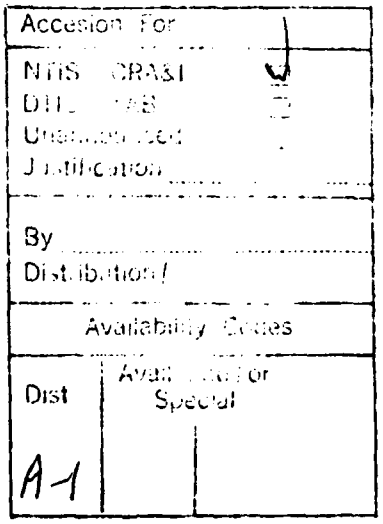

TABLE OF CONTENTS

A. Correlation between sera containing antibodies which recognize HIV-1 V3 based peptides in infected humans and neutralizing activity *in vitro*

This study examined the correlation between the presence of antibodies reactive with V3 based peptides in sera from infected individuals and the ability of these sera to neutralize homologous HIV-1 isolates *in vitro* (Warren et al., J. Virol. In Press). Since the V3 region of gp120 is frequently mentioned as a component in many potential AIDS vaccines, this type of correlation is vital in establishing the effectiveness of any potential V3 based vaccine. Sera from both the United States (n=37) and Tanzania (n=74) were examined. From the initial screening of sera against several V3 based peptides, it became apparent that the majority of sera from both the U.S. and Tanzania contained antibodies reactive with the MN based peptide (84% and 87% respectively). This suggests that the MN isolate is commonly found in both the U.S. and Tanzania. Surprisingly, a much smaller percentage of sera from the U.S. or Tanzania neutralized the MN isolate *in vitro* (16% and 47% respectively). In addition, we were unable to establish a correlation between anti-V3 peptide antibody titers and neutralization titers. Based on these results, we were unable to establish a correlation between the presence of V3 reactive antibodies in sera and its ability to neutralize homologous HIV-1 isolates *in vitro*. Thus, important HIV-1 neutralization epitopes appear to exist outside the principal neutralizing determinant and must be considered during the design of a successful AIDS vaccine.

B. Examination of hybrid peptides corresponding to the V3 region of HIV-1 gp120 for their ability to stimulate group specific neutralizing antibodies

The V3 loop region of gp120 consists of a conserved region near the top of the loop (GPGR) as well as conserved regions which flank the cysteine residues at the base of the loop. Two highly variable regions lie in between and appear to be immunodominant, since the majority of anti-V3 antibodies are type specific. We have constructed two hybrid peptides which correspond to the highly conserved regions of the V3 loop which maintain the integrity of the predicted secondary structure. Rabbits were immunized repeatedly with these peptides and then tested for the presence of group specific antibodies and neutralizing activity *in vitro*. A problem which arose during these rabbit immunizations was our inability to generate high antibody titers against the V3 peptides. While cross reactive antibodies were observed by ELISA in these sera to V3 peptides corresponding to HIV-1 isolates IIIB, RF and MN, only weak neutralization of these isolates was detected *in vitro*. The low antibody titers detected in these rabbits may reflect the weakly immunogenic nature of the conserved regions within the V3 loop of gp120.

C. Production of monoclonal antibodies against a composite peptide representing the V3 loop region of HIV-1 gp120

A composite peptide which represents conserved amino acids within the V3 loop of HIV-1 gp120 has been generated and has been used to immunize BALB/c mice. These mice are currently being used to produce monoclonal antibodies. We are in the process of screening hybrid clones for reactivity to the composite peptide. Since the V3 loop region of gp120 represents the principal neutralizing determinant, monoclonal antibodies reactive with this peptide may possess viral neutralizing activity as well.

D. Examination of anti-V3 antibodies in various mouse strains receiving multiple immunizations with recombinant gp160

We also examined the potential to induce group specific antibodies to the V3 region of gp120 in mice following repeated immunization with recombinant gp160 (rgp160) (Warren et al., submitted). Various inbred strains of mice (BALB/c, A/J, C57BL, CBA, DBA, and SJL) were immunized and tested in this study. Following immunization with rgp160, sera from these mice were examined for the presence of antibodies reactive with a panel of V3 based synthetic peptides which corresponded to HIV-1 isolates IIIB, RF, and MN. Our results suggested that for the majority of mouse strains, multiple immunizations with rgp160 resulted in the production of type specific antibodies to the V3 region of gp120.

E. Comparison of the humoral immune response to HIV-1 gp160 epitopes in sera from infected individuals from the U.S. and Tanzania

Differences among the epidemiologic patterns of HIV-1 infection in the U.S. and Africa suggest that viral isolates may exist in Africa which differ from those commonly found in the U.S. and Europe. These African isolates may possibly be more virulent and may differ in phenotypic makeup. We therefore examined the fine specificity of the humoral immune response in sera from both the U.S. and Tanzania to a panel of gp160 epitopes represented by synthetic peptides (Warren et al., J. Clin. Micro. 30:126, 1992; Nkya et al., J. Med. Virol. 37:61, 1992; Warren and Kennedy, AIDS Research Reviews, vol 3, In Press). Several differences in antibody fine specificity were observed between these two geographically distinct populations. For example, we observed that 91% of sera from infected individuals from the U.S. contained antibodies reactive with peptide 600-611, while only 50% of the Tanzanian sera were reactive. Significant differences in antibody reactivity to two other gp160 epitopes were also observed between sera from these two countries. These observations suggest that regional isolates of HIV-1 may exist throughout the world. This may be particularly important since potential AIDS vaccines which are comprised of

common U.S. or European viral isolates may not be effective against African isolates. Since HIV-1 isolates from Africa can be expected to eventually appear in the U.S., future vaccines will need to be effective against multiple isolates from throughout the world.

F. Isolation of HIV-1 from infected individuals from the United States

Recent studies have shown that while sera from HIV-1 infected individuals may contain antibodies which neutralize laboratory strains of HIV-1, this may have little correlation with disease progression. In fact, patients who have progressed to endstage AIDS frequently possess neutralizing antibodies in their sera. One possible explanation for this disparity may involve the adaptation of laboratory strains of HIV-1 to cell culture which may make these isolates more susceptible to *in vitro* neutralization. Through long term growth in cell culture these strains may alter their physiology to better adapt to these cell culture conditions, and therefore be less representative of the true field isolates of HIV-1. We are therefore obtaining primary HIV-1 isolates from peripheral blood lymphocytes (PBLs) of infected U.S. Military personnel. We are particularly interested in obtaining HIV-1 isolates from individuals who are clinically healthy as well as from those with more advanced stages of the disease. Isolates obtained from these two groups of individuals may potentially have different growth characteristics and/or phenotypic makeup. We currently have twelve such isolates in culture and are in the process of cloning them. Once cloned, sufficient quantities of these viral stocks will be grown up and frozen for future antibody reactivity and neutralization studies.

G. Isolation of HIV-1 from infected individuals from Tanzania

We have recently reported on the dissimilarities of anti-gp160 antibody fine specificities among infected individuals from the U.S. and Tanzania. Our study suggested that HIV-1 isolates from Tanzania may be phenotypically distinct from those commonly found in the U.S. and Europe. In addition, recent studies have indicated that while HIV-1 is transmitted primarily through male homosexuals and intravenous drug use in the U.S., transmission in African countries is primarily through heterosexual contact. Therefore, isolation and characterization of HIV-1 isolates from African countries could potentially be important for the understanding of these differences in epidemiologic patterns as well as the design of future AIDS vaccines. Viral isolates have been isolated from peripheral blood lymphocytes from two HIV-1 infected individuals from Tanzania (designated NKYA-1 and NKYA- 2). Sera from these two individuals were screened against several gp160 synthetic peptides. Sera from individual NKYA-1 was unreactive against V3 peptides corresponding to HIV-1 IIIB and MN isolates. In addition this sera was unreactive against a peptide corresponding to amino acids

600-611, a region which is highly conserved among sequenced isolates from the U.S. and Europe. In contrast, sera from individual NKYA-2 contained antibodies reactive against peptides corresponding to the V3 region of MN but not IIIB. This sera was also reactive against the 600-611 peptide. The two HIV-1 isolates obtained from these individuals also had quite different growth characteristics when placed in cell culture with SupT1 cells. For example, HIV-1 isolate NKYA-2 formed many large syncytia in culture and resulted in the death of the majority of SupT1 cells. Interestingly, SupT1 cells which are resistant to HIV-1 mediated cytolysis began growing in these cultures. These resistant cells have been maintained for several weeks and remain infected with the HIV-1 isolate NKYA-2. In contrast, HIV-1 isolate NKYA-1 formed few syncytia in culture and was not cytotoxic to the SupT1 cells. While capable of infecting the SupT1 cells, HIV-1 NKYA-1 appeared to die off in culture over a period of three weeks. We are currently in the process of establishing cell lines which will maintain the growth of each of these isolates *in vitro*.

In summary, we have isolated two HIV-1 isolates from Tanzanian patients which exhibit different growth characteristics *in vitro*. Antibodies in sera from these patients also demonstrate different anti-HIV-1 fine specificities suggesting that these isolates may differ in their phenotypic makeup. The primary isolates of these two Tanzanian strains which infected human PBMCs have been sent to Dr. Francine McCutchin (Henry Jackson Foundation, Rockville, MD) for envelope sequence determination.

H. Generation of anti-idiotypic antibodies against a Mab which inhibits CD4 binding to HIV-1 gp120

A human monoclonal antibody (designated 1171GP13) has been previously demonstrated to inhibit CD4 binding to HIV-1 gp120 and to neutralize both the HIV-1 SF2 and IIIB isolates. This Ab1 was originally isolated from EBV transformed B cells obtained from an HIV-1 infected patient. We are interested in generating monoclonal anti-idiotypic antibodies against this Ab1 in order to study the interactions between HIV-1 gp120 and the CD4 receptor. A group of mice have been immunized with the Ab1 preparation and are currently being used for hybridoma production. These clones are currently being screened for Ab1 reactivity. Positive clones will be characterized for their ability to inhibit Ab1 binding to gp120 and possible internal image characteristics.

I. Examination of peptide mimetics and regions of homology with HIV-1 gp120 and MHC molecules

We are also examining two peptides whose sequences correspond to regions of HIV-1 gp160 which contain structural homology with MHC class II molecules. The first peptide corresponds to a region of HIV-1 gp120 which appears to exhibit conformational homology

with human MHC class II DP molecules based on molecular modeling studies (Dalgleish, personal communication). The second peptide corresponds to a peptide mimetic of the conformation on the class II DP molecule which exhibits structural homology with HIV-1 gp120. Mice have been immunized with these peptides in order to generate monoclonal antibodies. Several fusions with these mice spleen cells have been done and hybridoma cultures are currently being screened for antibody reactivity to these peptides. These monoclonal antibodies will allow us to better characterize the interaction of these regions of HIV-1 gp160 with lymphocytes and monocytes.

Publications supported by this contract

1. Warren, R.Q., Wolf, H., Shuler, K., Eichberg, J.W., Zajac, R.A., Boswell, R.N., Kanda, P. and Kennedy, R.C. Synthetic peptides define the fine specificity of HIV-1 gp160 humoral immune response in HIV-1 infected chimpanzees. J. Virol. 64:486-492, 1990.

2. Warren, R.Q., Wolf, H., Zajac, R.A., Boswell, R.N., Kanda, P. and Kennedy, R.C. Patterns of antibody reactivity to selected HIV-1 gp160 epitopes in infected individuals grouped according to CD4+ cell levels. J. Clin. Immunol. 11:10-18, 1991.

3. Warren, R.Q., Nkya, W.M.M.M., Shao, J.F., Anderson, S.A., Wolf, H., Hendrix, C.W., Kanda, P., Wabuke, M., Boswell, R.N., Redfield, R.R., and Kennedy, R.C. Comparison of antibody reactivity to HIV-1 epitopes in sera from HIV-1 infected individuals from Tanzania and the United States. J. Clin. Micro. 30:126-131, 1992.

4. Nkya, W.M.M.M., Warren, R.Q., Wolf, H., Tesha, J., Redfield, R.R., Melcher, G.P., Burke, D.S., Kanda, P., and Kennedy, R.C. Fine specificity of the humoral immune response to HIV-1 gp160 in HIV-1 infected individuals from Tanzania. J. Med. Virol. 37:61-65, 1992.

5. Kennedy, R.C. Humoral immunity to HIV-1 gp160: Specificity analysis and anti-idiotype modulation. In Retroviruses des Cent Gardes (M. Girard and L. Valette, eds.). Lyon, Foundation Marcel Merieux, pp. 235-240.

6. Wolf, H., Warren, R.Q., Stunz, G.W., Shuler, K.R., Kanda, P. and Kennedy, R.C. Fine specificity of the murine antibody response to HIV-1 gp160 determined by synthetic peptides which define selected epitopes. Mol. Immunol. 29:989-998, 1992.

7. Nixon, A., Zaghouani, H., Penny, C., Lacroix, M., Dionne, G., Anderson, S.A., Kennedy, R.C., and Bona, C.A. The adjuvant effect of stearyl tyrosine on antibody responses to a synthetic peptide corresponding to peptide 503-535 from HIV-1 gp160. Viral Immunol. 5:141-150, 1992.

8. Warren, R.Q., and Kennedy, R.C. Fine specificity of the humoral immune response to HIV-1 gp160. In AIDS Research Reviews, Vol. 3 (W.C. Koff, F. Wong-Staal, R.C. Kennedy, eds). Marcel Dekker, New York, 1992 in press.

9. Warren, R.Q., Anderson, S.A., Nkya, W.M.M.M., Shao, J.F., Hendrix, C.W., Redfield, R.R., and Kennedy, R.C. Examination of sera from HIV-1 infected individuals for antibodies reactive with peptides corresponding to the principal neutralizing determinant of gp120 and *in vitro* neutralizing activity. J. Virol. 1992, in press.

10. Warren, R.Q., Wolf, H., Stunz, G.W., Kanda, P. and Kennedy, R.C. Antibody cross reactive to synthetic peptides representing the principal neutralizing determinant of HIV-1 varies among inbred strains of mice immunized with recombinant gp160. Submitted for publication.